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PROPERTIES OF MONOCLONAL ANTIBODIES TO STAPHYLOCOCCAL ENTEROTOXIN B

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ABSTRACT

Our laboratory has developed some forty monoclonal antibodies to staphylococcal enterotoxin B (SEB). Ten of these antibodies have been studied intensively to determine their fine specificity and biological activity. Preliminary data suggest that some of the antibodies protect mice from a lethal challenge with SEB. Some inhibit the SEB-induced proliferation of human or mouse cells. Others inhibit the binding of SEB to human MHC class II antigens. Because T cell and monocyte activation may be important factors in the intoxication caused by SEB, we hope that correlation of *in vitro* and *in vivo* studies will give insight regarding the mechanism of protection by antibodies.

INTRODUCTION

Staphylococcal enterotoxin B is a recognized threat agent that poses a danger to soldiers and civilians alike. It is necessary to have a vaccine, therapeutic agents, and a rapid detection capability for this toxin. The following studies were undertaken to gain an understanding of the mechanism by which antibodies protect against staphylococcal enterotoxins.

Passively-administered antibodies to SEB are known to confer protection of monkeys against *per os* (Bergdoll, 1966) or intravenous (Silverman *et al.* 1969) challenge with enterotoxin. Although it has not been proven that antibodies also protect against aerosolized SEB, immunity to aerosol challenge in immunized monkeys is correlated with antibody levels (Tseng *et al.* 1993).

The staphylococcal enterotoxins have recently been classified as "superantigens", a newly-identified group of antigens that stimulate T cells primarily according to the Vβ elements of their T cell receptors and do not need to be processed in order to be presented by antigen-presenting cells (reviewed by Herman *et al.*, 1991 and in special issues of Chemical Immunology (Vol. 55, 1992), Immunological Reviews (number 131, 1993) and Seminars in Immunology (Vol. 5 number 1, 1993)). Some of the enterotoxins are known to stimulate T cells and monocytes to make lymphokines and cytokines, which may cause or contribute to the pathology. T cells are the cells that are primarily responsible for the disease in at least two mouse models of enterotoxicosis (Marrack *et al.*, 1990; Miethke *et al.*, 1992), but recent work in mice suggests that there is a role for both T cells and cells of the monocyte-macrophage series in the pathogenesis of the disease (Grossman *et al.* 1990, 1992). There is a question as to how important T cells are in the response of monkeys to SEB.

In the present work, we have prepared monoclonal antibodies against SEB and have begun to characterize them with respect to their isotype, fine specificity, and *in vivo* and *in vitro* biological effects. It is hoped that this information will help in the design of effective vaccines against SEB.

MATERIALS AND METHODS

Production of hybridomas

BALB/c mice were immunized once with SEB and once with reduced, alkylated, heat-denatured SEB. The objective was to obtain antibodies against regions of SEB that are exposed in

the native molecule and those that are hidden. The spleen cells were fused with the X63.Ag 8.653 cell line and the cells from positive wells were cloned by limiting dilution. Some forty antibody-producing cell lines were obtained. Clones derived from ten of these cell lines were examined in detail. Before use in inhibition of binding of SEB to MHC class II, the antibodies were purified on protein A-Sepharose, protein G-Sepharose, or, in the case of one IgM antibody, by gel filtration on an AcA-34 (acrylamide-agarose) column.

Enzyme-linked immunosorbent assay

SEB (lot 14-30 from USAMRIID) was dissolved in carbonate-bicarbonate buffer and used to coat U-bottom polyvinyl chloride plates. Antibodies were detected with horseradish peroxidase-labeled rabbit anti-mouse or goat anti-mouse immunoglobulin reagents and the results were read on an ELISA plate reader.

Proliferation assay

Murine spleen cells at 5×10^5 per well in a volume of 0.2 ml were incubated for 3 days with 20 µg/ml of staphylococcal enterotoxin B. On day 3, ^{125}-I IUDR (iododeoxyuridine) was added, the cells were incubated for 18 hours and the incorporated radioactivity was counted in a scintillation counter.

Epitope Scanning

Overlapping peptides of 10-AA length from SEB residue 1 to 239 were synthesized on pins by Cambridge Research Biochemicals (Wilmington, DE), using the technique of Geysen *et al.* (1984). Antibodies were detected by horseradish peroxidase-labeled rabbit anti-mouse immunoglobulin.

Passive protection

Protection experiments were done using a mouse model of SEB toxicosis that was developed in our laboratory (James Yok-jen Chen, in progress; see Poster 106 in this session). Mice (C3H/HeJ, endotoxin hyporesponders) were injected with 1 ml of ascites fluid from one of the monoclonal antibodies. Two hours later, the mice were made sensitive to tumor necrosis factor and interleukin-1 by injecting them intraperitoneally with 20 µg/mouse of actinomycin D as described by Wallach *et al.* (1988). Twenty minutes later, they were challenged with monoclonal antibody and 50 µg per mouse of staphylococcal enterotoxin B intravenously.

Inhibition of SEP binding to MHC class II molecules

Biotinylated SEB at 1 μg/ml was incubated with an MHC class II-positive human T cell line, HuT-78 (American Type Culture Collection No. TIB 161). Various concentrations of purified monoclonal antibodies were incubated with biotinylated SEB before adding the SEB to the cells, and the biotinylated SEB was detected by incubating the cells with fluorescein-avidin or phycoerythrin-avidin and examining the cells in a flow cytometer.

RESULTS

Monoclonal antibodies

The fusions producing antibodies against SEB were identified by ELISA and the cells were cloned by limiting dilution. The antibodies were then tested by ELISA against a panel of staphylococcal enterotoxins to determine their cross-reactions.

TABLE 1

Properties of anti-SEB monoclonal antibodies

Monoclonal Antibody	Isotype	Other SE's Recognized	Passive Protection live/total*
1	IgA	A, C_1, D, E, T	0/5
2	IgG1	none	0/4
3	IgG1	SEC_1	n.d.
4	IgG1	SEC_1	n.d.
5	IgG	none	n.d.
6	IgG1	none	n.d.
7	IgG1	none	n.d.
8	IgG1	A, C_1, D, E, T	n.d.
9	IgM	SEC_1	5/5
10	IgG2a	none	0/5
11	IgG1	none	n.d.
12	IgG	none	n.d.

n.d. = not done

* Passive protection data represent the number of mice alive eight days after challenge. The control group received no antibody; 3 of eight mice in this group survived to day eight. Usually all mice treated in this manner die.

As shown in Table 1, two of the antibodies reacted with SEB only, while two others reacted with SEA, SEB, SEC_1, SED, SEE, and TSST-1. The rest fell in the middle, reacting with some enterotoxins but not others.

One of the monoclonal antibodies was shown by the pepscan technique of Geysen and coworkers (1984) to be directed against a peptide with its amino terminus at residue 180 of SEB. Out of the 12 antibodies that have been most intensively studied, 10 were IgG. Of the IgG antibodies for which a subclass is known, 6 are IgG1 and one is IgG2a (Table 1).

T cell proliferation

Culture supernatants from ten of the monoclonal antibodies were tested for their ability to inhibit proliferation of murine spleen cells and human peripheral blood mononuclear cells. Three of the antibodies showed significant inhibition of murine spleen cell proliferation, and four showed inhibition of human mononuclear cell proliferation (Table 2).

TABLE 2

Properties of anti-SEB monoclonal antibodies (continued)

Monoclonal Antibody	Inhibition of Class II Binding	Inhibition of Murine Spleen Cell Proliferation	Inhibition of Human Mononuclear Cell Proliferation
1	0	0	0
2	+	0	+
3	0	0	0
4	0	+	+
5	n.d	0	0
6	n.d.	+	++
7	n.d.	0	0
8	n.d.	+	+
9	0	0	0
10	0	0	0
11	0	n.d.	n.d.
12	n.d.	n.d.	n.d.

"+" = Proliferation less than 50% of control
"++" = proliferation less than 25% of control

Three of the antibodies inhibited both murine and human cell proliferation. A control antibody directed against an irrelevant antigen did not inhibit proliferation of cells of either species.

Inhibition of SEB binding to MHC class II molecules

Only one of seven antibodies tested was able to inhibit binding of biotinylated SEB to MHC class II antigens on HuT-78 cells (Table 2).

Passive protection

The results of the passive protection study are preliminary. One of the monoclonal antibodies is very promising (Table 1).

DISCUSSION

Passively-transferred antibodies can protect monkeys from gastric or intravenous challenge with SEB (Bergdoll, 1966; Silverman *et al.*, 1969). A microsphere-encapsulated SEB toxoid vaccine candidate provided protection against challenge, and the level of that protection was correlated with anti-SEB antibody levels in the plasma (Tseng *et al.*, 1993). We have preliminary evidence that passively-administered antibodies can protect mice from intravenous challenge with SEB. In order to develop a vaccine that can protect against SEB, it may be important to know how antibodies accomplish this protection. We have used assays designed to test *in vitro* responses that correspond to two proposed mechanisms of action of staphylococcal enterotoxins, i.e., T cell activation and MHC class II binding. We have an antibody that inhibits binding to human MHC class II antigens and several antibodies that inhibit mononuclear cell proliferation (presumably the responding cells are T cells). We also have antibodies that might confer various levels of protection *in vivo*. However, we have seen no correlation between these various activities. It is possible that a larger panel of antibodies will reveal such a correlation, or that the relevant activity of a protective antibody is to inhibit another type of *in vivo* response, such as binding to mast cells, lung or kidney cells, or other unknown cells. It may be that an antibody that inhibits binding to human MHC class II antigens will not inhibit binding to murine MHC class II. We are continuing our binding studies with murine class II antigens. Finally, it is possible that the most important action of antibodies to SEB is to facilitate the clearance of SEB from the blood stream, and that *in vitro* assays will not allow us to predict which antibodies will

protect *in vivo* and which antibodies will not.

We believe that the antibodies that react with SEB alone may be useful in detection of SEB. Perhaps they can be used in the newly-described immuno-PCR technique (Sano *et al.*, 1992), which is likely to be several orders of magnitude more sensitive than the enzyme-linked immunosorbent assay.

The existence of antibodies that react with several of the enterotoxins agrees with the results of several other laboratories (Meyer *et al.*, 1984; Lapeyre *et al.*, 1987; Shinagawa *et al.*, 1991; and Goyache *et al.*, 1992), and it suggests that it will be possible to make a polyvalent vaccine, i.e., one that protects against several enterotoxins. This is necessary because any weaponized toxin will likely be "dirty", containing a poorly defined mixture of toxins. In addition, all nine known enterotoxin genes have been cloned (the cloning of SEF was reported by Ren *et al.*, 1993; the cloning of SEG was reported by Betley *et al.*, 1992), making it relatively easy to make any of the toxins in large quantities.

Antibodies that protect mice against an SEB challenge might also protect other species against SEB challenge. "Humanized" murine antibodies (antibodies with human amino acid sequences except that they have variable regions or complementarity-determining regions obtained from murine antibodies) show some promise for a variety of conditions and are now in clinical trials (Harris and Emery, 1993).

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